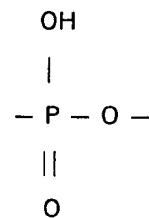


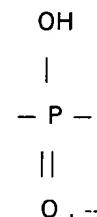
Dean L. Engelhardt, et al.
Serial No.: 08/479,997
Filed: June 7, 1995
Page 3 (Amendment Under 37 C.F.R. § 1.116 – November 20, 1998)



-- 457. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said covalent attachment is selected from the group consisting of



and



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-- 458. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal. --

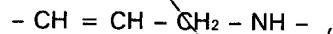
Sig

-- 459. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a $-\text{CH}_2\text{NH}-$ moiety, or both. --

-- 460. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises an allylamine group. --



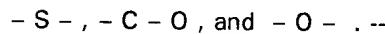
-- 461. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to the nucleotide, or any of the moieties:



OH,

O

||



-- 462. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage of Sig includes a glycosidic linkage moiety. --

-- 463. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said PM is mono-, di or tri-phosphate and said Sig moiety is covalently attached to said PM through a phosphorus or phosphate oxygen. --

-- 464. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing. --

-- 465. (NEW) The oligo- or polydeoxyribonucleotide of claim 464, wherein said electron dense component comprises ferritin. --

Sig
-- 466. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin. --

-- 467. (NEW) The oligo- or polydeoxyribonucleotide of claim 464, wherein said magnetic component comprises magnetic oxide. --

-- 468. (NEW) The oligo- or polydeoxyribonucleotide of claim 467, wherein said magnetic oxide comprises ferric oxide. --

-- 469. (NEW) The oligo- or polydeoxyribonucleotide of claim 464, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β -galactosidase, ribonuclease, glucose oxidase and peroxidase. --

-- 470. (NEW) The oligo- or polydeoxyribonucleotide of claim 464, wherein said metal-containing component is catalytic. --

F
-- 471. (NEW) The oligo- or polydeoxyribonucleotide of claim 464, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl. --

-- 472. (NEW) The oligo- or polydeoxyribonucleotide of claim 464, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten. --

-- 473. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide. --

-- 474. (NEW) A composition comprising the oligo- or polydeoxyribonucleotide of claim 454, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed. --

-- 475. (NEW) The composition of claim 474, wherein said polypeptide comprises polylysine. --

475
-- 476. (NEW) The composition of claim 474, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin. --

-- 477. (NEW) The composition of claim 474, wherein Sig is a ligand and said polypeptide is an antibody thereto. --

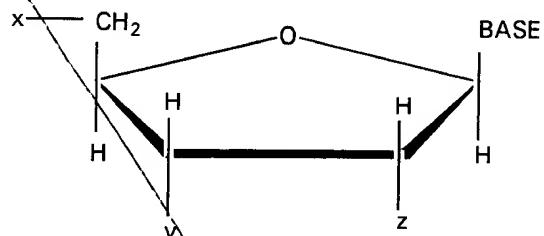
-- 478. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide. --

478
-- 479. (NEW) The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has hydrogens at the 2' position thereof. --

479
-- 480. (NEW) The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has hydrogens at each of the 2' and 3' positions thereof. --

-- 481. (NEW) The oligo- or polydeoxyribonucleotide of claim 454, comprising at least one ribonucleotide. --

-- 482. (NEW) An oligo- or polydeoxribonucleotide comprising at least one nucleotide having the structural formula:



b6 b7c
wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H-, HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H-, HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

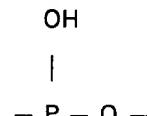
wherein z is H-; and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z. --

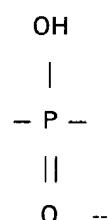
-- 483. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig is or renders the nucleotide or the oligo- or ~~polydeoxyribonucleotide~~ ^{polydeoxyribonucleotide} ~~polydeoxyribonucleotide~~ self-signaling or self-indicating or self-detecting. --

-- 484. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety comprises at least three carbon atoms. --

-- 485. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said covalent attachment is selected from the group consisting of



and

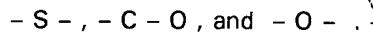
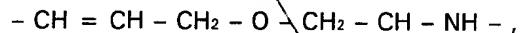
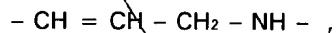
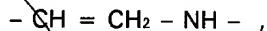


F1
-- 486. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal. --

SuM
-- 487. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a $-\text{CH}_2\text{NH}-$ moiety, or both. --

-- 488. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises an allylamine group. --

-- 489. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to x, y or z, or any of the moieties:



-- 490. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage of Sig includes a glycosidic linkage moiety. --

-- 491. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said x and y each comprise a member selected from the group consisting of mono-, di or tri-phosphate and said Sig moiety is covalently attached to either or both of said x and y through a phosphorus or phosphate oxygen. --

-- 492. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing. --

-- 493. (NEW) The oligo- or polydeoxyribonucleotide of claim 492, wherein said electron dense component comprises ferritin. --

Sub P11
-- 494. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin. --

-- 495. (NEW) The oligo- or polydeoxyribonucleotide of claim 492, wherein said magnetic component comprises magnetic oxide. --

-- 496. (NEW) The oligo- or polydeoxyribonucleotide of claim 495, wherein said magnetic oxide comprises ferric oxide. --

-- 497. (NEW) The oligo- or polydeoxyribonucleotide of claim 492, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β -galactosidase, ribonuclease, glucose oxidase and peroxidase. --

T
-- 498. (NEW) The oligo- or polydeoxyribonucleotide of claim 492, wherein said metal-containing component is catalytic. --

-- 499. (NEW) The oligo- or polydeoxyribonucleotide of claim 492, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl. --

-- 500. (NEW) The oligo- or polydeoxyribonucleotide of claim 492, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten. --

-- 501. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide. --

-- 502. (NEW) A composition comprising the oligo- or polydeoxyribonucleotide of claim 482, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed. --

-- 503. (NEW) The composition of claim 500, wherein said polypeptide comprises polylysine. --

Su b n 1/2
-- 504. (NEW) The composition of claim 502, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin. --

-- 505. (NEW) The composition of claim 502, wherein Sig is a ligand and said polypeptide is an antibody thereto. --

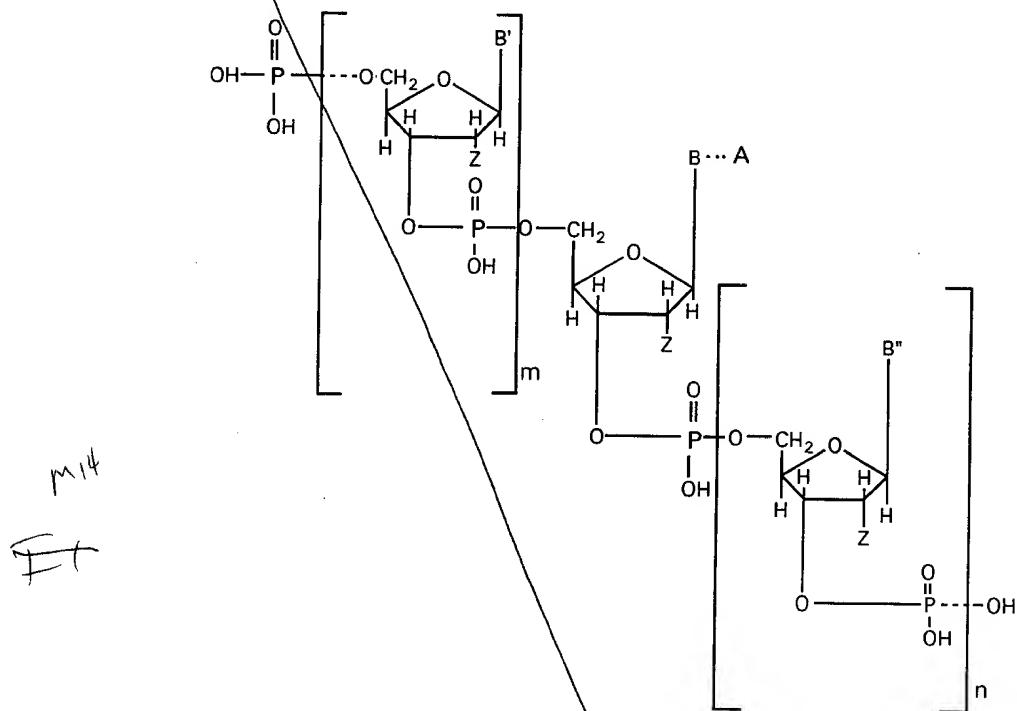
-- 506. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide. --

T (-- 507. (NEW) The oligo- or polydeoxyribonucleotide of claim 506, wherein z of said terminal nucleotide comprises a hydrogen at the 2' position thereof. --

Su b n 3
-- 508. (NEW) The oligo- or polydeoxyribonucleotide of claim 506, wherein both y and z of said terminal nucleotide comprise a hydrogen at each of the 3' and 2' positions thereof, respectively. --

-- 509. (NEW) The oligo- or polydeoxyribonucleotide of claim 482, comprising at least one ribonucleotide. --

-- 510. (NEW) The oligo- or polydexoxyribonucleotide of claim 482, having the structural formula:



wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula. --

-- 511. (NEW) An oligo- or polyribonucleotide comprising at least one ribonucleotide having the formula

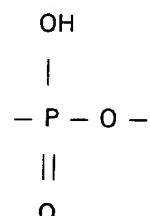
Sig – PM – SM - BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM at a position of SM selected from the 2', 3' and 5' positions, or combinations thereof, said BASE being attached to SM, and Sig being covalently attached to PM directly or via a chemical linkage, said Sig being a moiety capable of non-radioactive detection when attached to PM or when said nucleotide is incorporated into said oligo- or polyribonucleotide, provided that when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to said oligo- or polyribonucleotide. --

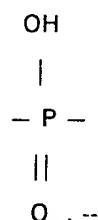
My -- 512. (NEW) The oligo- or polyribonucleotide of claim 511, wherein said Sig is or renders the nucleotide self-signaling or self-indicating or self-detecting. --

polyribonucleotide
-- 513. (NEW) The oligo- or *polyribonucleotide* of claim 511, wherein said Sig moiety comprises at least three carbon atoms. --

polyribonucleotide
-- 514. (NEW) The oligo- or *polyribonucleotide* of claim 511, wherein said covalent attachment is selected from the group consisting of



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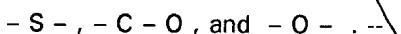
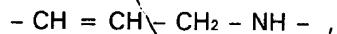
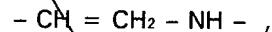


polyribonucleotide
-- 515. (NEW) The oligo- or *polyribonucleotide* of claim 511, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal. --

sym -- 516. (NEW) The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a $-\text{CH}_2\text{NH}-$ moiety, or both. --

polyribonucleotide
-- 517. (NEW) The oligo- or *polyribonucleotide* of claim 511, wherein said chemical linkage comprises an allylamine group. --

-- 518. (NEW) The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to the nucleotide, or any of the moieties:



II

polyribonucleotide

-- 519. (NEW) The oligo- or *polyribonucleotide* of claim 511, wherein said chemical linkage of Sig includes a glycosidic linkage moiety. --

Polynucleotide

-- 520. (NEW) The oligo- or *polyribonucleotide* of claim 511, wherein said PM is mono-, di- or tri-phosphate and said Sig moiety is covalently attached to said PM through a phosphorus or phosphate oxygen. --

polyribonucleotide

-- 521. (NEW) The oligo- or *polyribonucleotide* of claim 511, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing. --

polyribonucleotide

-- 522. (NEW) The oligo- or *polyribonucleotide* of claim 521, wherein said electron dense component comprises ferritin. --

M17

-- 523. (NEW) The oligo- or polyribonucleotide of claim 511, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin. --

-- 524. (NEW) The oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 521, wherein said magnetic component comprises a magnetic oxide. --

-- 525. (NEW) The oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 524, wherein said magnetic oxide comprises ferric oxide. --

-- 526. (NEW) The oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 521, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β -galactosidase, ribonuclease, glucose oxidase and peroxidase. --

F1

-- 527. (NEW) The oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 521, wherein said metal-containing component is catalytic. --

-- 528. (NEW) The oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 521, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl. --

-- 529. (NEW) The oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 521, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten. --

-- 530. (NEW) The oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 511, wherein said oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} is terminally ligated or attached to a polypeptide. --

-- 531. (NEW) A composition comprising the oligo- or ~~polyribonucleotide~~ ^{Poly nucleotide} of claim 511, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed. --

-- 532. (NEW) The composition of claim 531, wherein said polypeptide comprises polylysine. --

MA
-- 533. (NEW) The composition of claim 531, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin. --

-- 534. (NEW) The composition of claim 531, wherein Sig is a ligand and said polypeptide is an antibody thereto. --

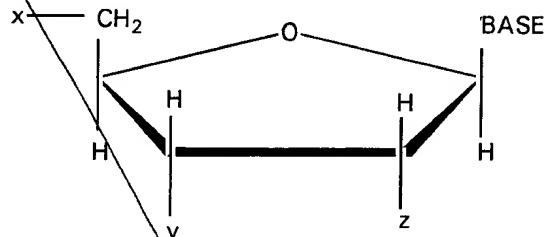
-- 535. (NEW) The oligo- or polyribonucleotide of claim 511, wherein said Sig moiety is attached to a terminal ribonucleotide in said oligo- or polyribonucleotide. --

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-- 536. (NEW) The oligo- or polyribonucleotide of claim 535, wherein the sugar moiety of said terminal ribonucleotide has a hydrogen atom at the 2' position thereof. --

-- 537. (NEW) The oligo- or polyribonucleotide of claim 535, wherein the sugar moiety of said terminal nucleotide has a hydrogen atom at each of the 2' and 3' positions thereof. --

Polyribonucleotide
-- 538. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 511, comprising at least one deoxyribonucleotide. --

-- 539. (NEW) An oligo- or polyribonucleotide comprising at least one nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H-, HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

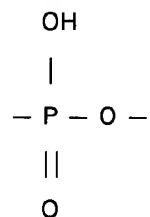
wherein z is HO-; and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z, provided that when Sig is attached through a chemical linkage to y of a terminal ribonucleotide, said chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to said oligo- or polyribonucleotide. --

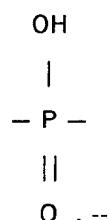
-- 540. (NEW) The oligo- or ~~polyribonucleotide~~ ^{polynucleotide} of claim 539, wherein said Sig is or renders the nucleotide or the oligo- or polynucleotide self-signaling or self-indicating or self-detecting. --

-- 541. (NEW) The oligo- or ~~polyribonucleotide~~ ^{polynucleotide} of claim 539, wherein said Sig moiety comprises at least three carbon atoms. --

polyribonucleotide
-- 542. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 539, wherein said covalent attachment is selected from the group consisting of



and



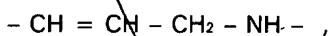
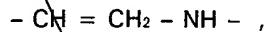
F1

polyribonucleotide
-- 543. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 539, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal. --

polyribonucleotide
-- 544. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 539, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a $-\text{CH}_2\text{NH}-$ moiety, or both. --

polyribonucleotide
-- 545. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 539, wherein said chemical linkage comprises an allylamine group. --

-- 546. (NEW) The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to x, y or z, or any of the moieties:



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Polyribonucleotide

-- 547. (NEW) The oligo- or *Polyribonucleotide* of claim 539, wherein said chemical linkage of Sig includes a glycosidic linkage moiety. --

Polyribonucleotide

-- 548. (NEW) The oligo- or *Polyribonucleotide* of claim 539, wherein said x and y each comprise a member selected from the group consisting of mono-, di or tri-phosphate and said Sig moiety is covalently attached to either or both of said x and y through a phosphorus or phosphate oxygen. --

Polyribonucleotide

-- 549. (NEW) The oligo- or *Polyribonucleotide* of claim 539, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing. --

Polyribonucleotide

-- 550. (NEW) The oligo- or *Polyribonucleotide* of claim 549, wherein said electron dense component comprises ferritin. --

by M
-- 551. (NEW) The oligo- or polyribonucleotide of claim 539, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin. --

-- 552. (NEW) The oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 549, wherein said magnetic component comprises magnetic oxide. --

-- 553. (NEW) The oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 552, wherein said magnetic oxide comprises ferric oxide. --

-- 554. (NEW) The oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 549, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β -galactosidase, ribonuclease, glucose oxidase and peroxidase. --

F1
-- 555. (NEW) The oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 549, wherein said metal-containing component is catalytic. --

-- 556. (NEW) The oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 549, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl. --

-- 557. (NEW) The oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 549, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten. --

-- 558. (NEW) The oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 539, wherein said oligo- or ~~polyribonucleotide~~ *Polynucleotide* is terminally ligated or attached to a polypeptide. --

-- 559. (NEW) A composition comprising the oligo- or ~~polyribonucleotide~~ *Polynucleotide* of claim 539, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed. --

-- 560. (NEW) The composition of claim 559, wherein said polypeptide comprises polylysine. --

M24
-- 561. (NEW) The composition of claim 559, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin. --

-- 562. (NEW) The composition of claim 559, wherein Sig is a ligand and said polypeptide is an antibody thereto. --

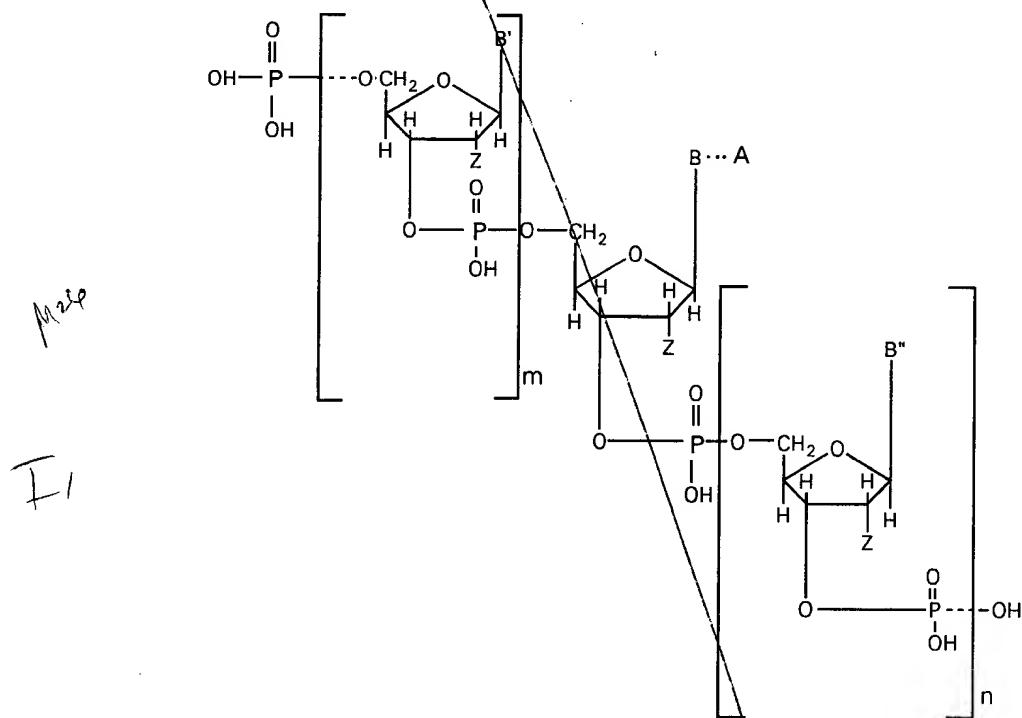
-- 563. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 539, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or ~~polyribonucleotide~~. --

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M25
-- 564. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 563, wherein z of said terminal nucleotide comprises a hydrogen at the 2' position thereof. --

-- 565. (NEW) The oligo- or polyribonucleotide of claim 563, wherein both y and z of said terminal nucleotide comprise a hydrogen at each of the 3' and 2' positions thereof, respectively. --

M25
-- 566. (NEW) The oligo- or ~~polyribonucleotide~~ of claim 539, comprising at least one deoxyribonucleotide. --

-- 567. (NEW) The oligo- or polyribonucleotide of claim 539, having the structural formula:



wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula. --

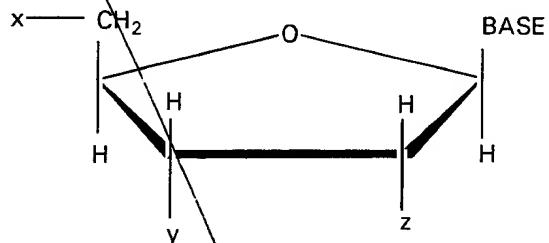
-- 568. (NEW) A composition comprising a polymeric compound having attached directly or indirectly thereto at least one deoxyribonucleotide having the formula:

Sig – PM – SM - BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM, said BASE being attached to SM, and Sig being covalently attached to PM directly or via a chemical linkage, said Sig being a moiety capable of non-radioactive detection when attached to PM or when said deoxyribonucleotide is incorporated into said composition. --

-- 569. (NEW) The composition of claim 568, wherein said polymeric compound is selected from the group consisting of an oligo- or polynucleotide, an oligo- or polypeptide, and an oligo- or polysaccharide. --

-- 570. (NEW) A composition comprising a polymeric compound attached directly or indirectly to at least one deoxyribonucleotide having the structural formula:



wherein BASE is selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x comprises a member selected from the group consisting of: H-, HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y comprises a member selected from the group consisting of: H-, HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein z comprises H-; and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z. --

-- 571. (NEW) The composition of claim 570, wherein said polymeric compound is selected from the group consisting of an oligo- or polynucleotide, an oligo- or polypeptide, and an oligo- or polysaccharide. --

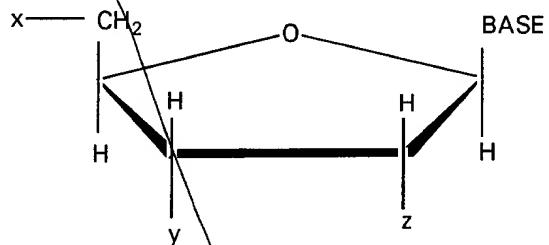
-- 572. (NEW) A composition comprising a polymeric compound having attached directly or indirectly thereto at least one ribonucleotide having the formula:

$\text{Sig} - \text{PM} - \text{SM} - \text{BASE}$

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM at a position of SM selected from 2', 3' and 5', or combinations thereof, said BASE being attached to SM, and Sig being covalently attached to PM directly or via a chemical linkage, said Sig being a moiety capable of non-radioactive detection when attached to PM or when said ribonucleotide is incorporated into said composition, provided that when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to said composition. --

-- 573. (NEW) The composition of claim 572, wherein said polymeric compound is selected from the group consisting of an oligo- or polynucleotide, an oligo- or polypeptide, and an oligo- or polysaccharide. --

-- 574. (NEW) A composition comprising a polymeric compound having attached directly or indirectly thereto at least one nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H-, HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of HO-, a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein z is HO-; and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z, provided that when Sig is attached through a chemical linkage to y of a terminal ribonucleotide, said chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to said composition. --

-- 575. (NEW) The composition of claim 572, wherein said polymeric compound is selected from the group consisting of an oligo- or polynucleotide, an oligo- or polypeptide, and an oligo- or polysaccharide. --

* * * * *

polydeoxyribonucleotide. To illustrate this point, Applicants have tracked the new claims (454-575) against previously pending claims.

<u>New Claim No.</u>	<u>Previous Claim No.</u>
454, 482, 511, 539	310, 405 (bifurcated)
455, 483, 512, 540	311
456, 484, 513, 541	312
457, 485, 514, 542	313, 314
458, 486, 515, 543	315
459, 487, 516, 544	316
460, 488, 517, 545	317
461, 489, 518, 546	318
462, 490, 519, 547	319
463, 491, 520, 548	320
464, 492, 521, 549	321
465, 493, 522, 550	322
466, 494, 523, 551	323
467, 495, 524, 552	325
468, 496, 525, 553	326
469, 497, 526, 554	328
470, 498, 527, 555	330
471, 499, 528, 556	332
472, 500, 529, 557	334
473, 501, 530, 558	302
474, 502, 531, 559	303
475, 503, 532, 560	304
476, 504, 533, 561	305
477, 505, 534, 562	306
478, 506, 535, 563	335
479, 507, 536, 564	336
480, 508, 537, 565	337
481, 509, 538, 566	309
510, 567	432
568, 570, 572, 574	433, 451-453 (bifurcated)
569, 571, 573, 575	450

Lastly, Applicants and their attorney would like to point out that a sincere effort was made to limit the number of the new claims to a number as close as possible to 122, which is the same number as was last presented by their July 6, 1998 Amendment Under 37 C.F.R. § 1.115. As might be expected with bifurcation of the subject matter into roughly two divisions, either the total number of claims or the total number of independent claims would have increased significantly. In this instance, however, no additional claims are being presented, keeping the total number at 122, and the number of independent claims is no greater than the highest number of independent claims previously presented in this application, that is to say, six. In effect, there is practically no increase in the number of claims.

The Rejection Under 35 U.S.C. §112, First Paragraph

Claims 310-372 and 405-453 stand rejected for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner's remarks in that rejection are set forth on pages 2 and 3 in the September 29, 1998 Office Action.

The rejection for inadequate description is respectfully traversed.

For the sake of completeness and as a followup to their November 3, 1998 interview, Applicants would like to summarize below the principal points on the issue of adequate description all of which were set forth in their earlier filed responses.¹

¹ See for example, Applicants' November 24, 1997 Amendment Under 37 C.F.R. § 1.115 (page 39, through page 40, 2nd full ¶); see also Declaration of Dr. Dean L. Engelhardt In Support of Adequate Description and Enablement (page 8, indented portion bottom of page, through page 12, 1st ¶) referenced in Applicants' November 24, 1997 Amendment. See also Applicants' July 6, 1998 Amendment Under 37 C.F.R. § 1.116 (footnote 1, pages 7-8).

1. EXPLICIT DISCLOSURE OF PHOSPHATE MOIETY LABELED NUCLEIC ACIDS

<u>Citation in the Specification</u>	<u>Description</u>
page 94, last ¶, thru page 95, 1st ¶;	Still further, nucleotides in accordance with the practices of this invention include the nucleotides having the formula, $\begin{array}{c} \text{Sig} \\ \\ \text{P} - \text{S} - \text{B} \dots \end{array}$
page 96, thru page 98, 1st ¶;	... The nucleotides are then modified in accordance with the practices of this invention by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig .
page 103, 1st full ¶; and	The special nucleotides of this invention and polynucleotides including such nucleotides, either single-stranded or double-stranded polynucleotides, DNA and/or RNA, comprising the components, phosphoric acid moiety P, the sugar or monosaccharide moiety S, the base moiety B, a purine or pyrimidine, and the signalling or self-detecting moiety, Sig, covalently attached to either the P, S or B moieties , ...
page 103, last ¶, continuing thru page 106, 1st ¶	... nucleotides in accordance with this invention containing the above-described components P, S, B and Sig , are suitably prepared ... [bold added above]

2. OTHER EXPLICIT DISCLOSURE OF PHOSPHATE MOIETY LABELED NUCLEIC ACIDS

<u>Citation in the Specification</u>	<u>Description</u>
page 90, last ¶	... and a signalling chemical moiety Sig covalently attached thereto, either to the P, S or B moiety .
page 93, 1st ¶	... include a chemical moiety Sig covalently attached to the P, S and/or B moieties .
page 96, 1st ¶	... by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig .

page 98, 1st ¶

. . . the **Sig** component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm . . .

page 103, 1st full ¶

. . . and the signalling or self-detecting moiety, **Sig**, covalently attached to either the P, S or B moieties, as indicated hereinabove, . . .

page 104, 1st ¶

. . . nucleotides in accordance with this invention containing the above-described components P, S, B and **Sig**, . . .

page 105, 1st ¶

. . . the nucleotides of this invention include the P, S, B and **Sig** components wherein the **Sig** is covalently attached to either the P, S or B moieties

page 105, 2nd ¶

The moiety **Sig** attached to the special nucleotides of this invention containing the other moieties or components P, S, B provides a site per se for the attachment thereto, the **Sig** component, . . .

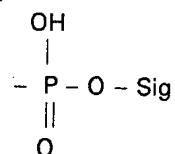
page 106, 1st ¶

. . . the special P, S, B and **Sig**-containing nucleotides of this invention, including polynucleotides containing such nucleotides . . .
[bold added above]

3. EXPLICIT DISCLOSURE IN THE ORIGINALLY FILED CLAIMS

originally filed claim 143

A nucleotide having the general formula P-S-B wherein P is the phosphoric acid moiety, S the sugar or monosaccharide moiety and B the base moiety, said nucleotide having covalently attached to the P or S or B moiety a chemical moiety **Sig**, said **Sig** chemical moiety when attached to the P moiety is attached thereto via the chemical linkage,



and when **Sig** is attached to the S moiety, the S moiety is a ribose group, said chemical moiety **Sig** when attached to said P, S or B being capable of signalling itself or makes itself self-detecting or its presence known.
[bold added above]

Dean L. Engelhardt, *et al.*

Serial No.: 08/479,997

Filed: June 7, 1995

Page 32 (Amendment Under 37 C.F.R. § 1.116 – November 20, 1998)

In view of the previously submitted information as summarized above, Applicants respectfully request reconsideration and withdrawal of the rejection for inadequate description under 35 U.S.C. §112, first paragraph.

The Objection and Rejection Under 35 U.S.C. §112, First Paragraph

Claims 310-372 and 405-453 stand rejected for nonenablement under 35 U.S.C. §112, first paragraph. The Examiner's remarks in the enablement rejection are given on page 3 of the Office Action.

The enablement rejection is respectfully traversed.

Again, for the sake of completeness and to follow up on the discussions held at the November 3, 1998 interview, Applicants have summarized on the next page the principal points on the enablement issue, at least a couple of which were brought to the Examiner's attention in the first instance.

<u>Disclosure/Citation in the Specification</u>	<u>Comments</u>
Example IV (page 56)	end labeling of oligodeoxyribonucleotides with terminal transferase
Example V (page 57)	provides means for labeling the oxygen or phosphorus of the phosphate moiety of a nucleotide, including the 5' phosphate, in an oligo- or polynucleotide using procedure of Halloran and Parker, <u>J. Immunol.</u> 96:373 (1966)
RNA ligase (page 20, 1st ¶)	phosphate labeling (attaching Sig to the 5' phosphate)
terminal transferase (page 99, last ¶ thru page 100)	attaching Sig to the 3' end of a nucleic acid
DNA ligase reaction (page 101, 1st ¶)	phosphate labeling (attaching Sig to the 3' phosphate)

² Applicants wish to eagerly point out that in Example V, the cited 1966 Halloran and Parker article actually discloses in Figure 1 on page 374, two separate reaction procedures for modifying the 5' phosphate of a nucleotide (either DNA or RNA). The first reaction procedure, designated Reaction 1 in Figure 1, involves formation of a phosphodiester bond with protein seryl and threonyl residues. The second reaction procedure, designated Reaction 2, involves N-P bond formation with protein epsilon-amino groups. Figure 1 from Halloran and Parker is reproduced below:

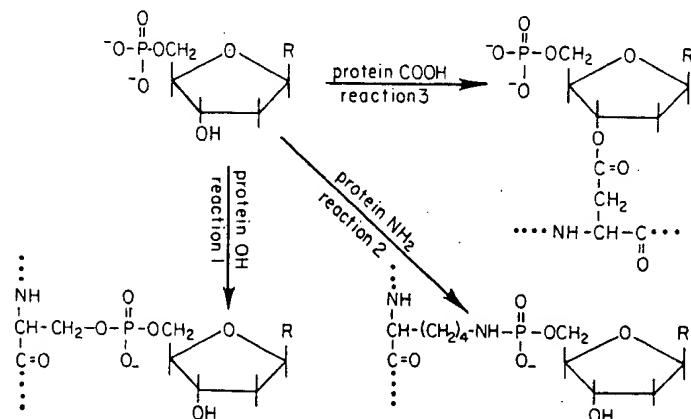


Figure 1. Possible reactions of nucleotides with proteins in the presence of carbodiimides. Very unstable products such as acyl phosphates are not shown.

In view of the foregoing information and remarks, Applicants respectfully request reconsideration and withdrawal of the enablement rejection.

The Rejection Under 35 U.S.C. §103

Claims 310-372 and 405-453 stand rejected under 35 U.S.C. §103 for being unpatentable over Gohlke et al., U.S. Patent No. 4,378,458, filed on March 30, 1981 in view of Sodja et al., Nucleic Acids Research 5(2):385-401 (1978) and further in view of Applicants' admissions. The Examiner's remarks are set forth on pages 4-6 in the Office Action.

The obviousness rejection is respectfully traversed.

As indicated in the opening remarks above, Applicants have bifurcated the subject matter of their present invention into oligo- or polydeoxyribonucleotides (claims 454-508 and 568-571) and oligo- or polyribonucleotides (claims 511-567 and 572-575). The former set of claims directed to the polydeoxynucleotides, contain similar language as was presented in the previously pending claims. The latter set of claims directed to the polyribonucleotides contain the provision that "when Sig is attached through a chemical linkage to a terminal phosphate (PM in the case of claims 511 and 572; y in the case of claims 539 and 574) at the 3' position of a terminal ribonucleotide, said chemical linkage is not derived from a ribonucleotide previously attached to said oligo- or polyribonucleotide."

Turning to the polydeoxynucleotide claims first, claims 454-508 and 568-571, it is respectfully submitted that the cited documents, Gohlke et al. in view of Sodja et al., do not either in combination or singly, render these claims obvious for at least two very significant reasons.

First, Sodja's disclosure is limited to RNA and it does not disclose or suggest that DNA could be similarly disrupted by the periodate oxidation chemistry employed to break open the ribose sugar aldehyde ring, or by any other chemistry for that matter.

Second, Sodja's periodate chemistry can only be applied to a terminal ribonucleotide in RNA. The periodate oxidation chemistry disclosed in Sodja and Davidson requires two 2', 3' OH groups on the ribose aldehyde in the *cis* configuration. In the case of polydeoxyribonucleotides in which two hydrogens are present at the 2' position (meaning that a hydroxyl group is not present or available for oxidation), Sodja's periodate chemistry cannot be carried out. In effect, Sodja's disclosure is nonenabling with respect to *DNA* chemistry and polydeoxyribonucleotides. Thus, a person of ordinary skill in the art would have been overtly discouraged to look to Sodja's disclosure for the purpose of labeling the phosphate moiety in DNA. And Gohlke's patent, which admittedly discloses a number of labels suitable for immunoassays, would not have cured the deficiencies in Sodja's chemistry.

In the case of Applicants' polyribonucleotide claims, the cited documents do not reach the subject matter of claims 511-567 and 572-575 because no teaching or suggestion is offered for labeling nonradioactively the phosphate moiety of a polyribonucleotide directly at the 5' position or indirectly using anything other than a terminal ribonucleotide in which the ribose ring is disrupted and broken at the 2', 3' position through the periodate chemistry. Moreover, neither Sodja *et al.* nor Gohlke *et al.* teach or suggest that the phosphate moiety at any other position, such as the 2' or 3' positions, could or even should be labeled nonradioactively as set forth in the present invention.

In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the obviousness rejection, thereby placing each of the new claims, 454-575, in allowable condition. Favorable passage to allowance is respectfully urged.

Request Under 37 C.F.R. § 1.129(a) To Withdraw
Finality Of Previous Office Action

Concurrently with the filing of this Amendment, Applicants are making a request under 37 C.F.R. § 1.129(a) to withdraw the finality of the September 29, 1998 Office Action. Authorization for the fee in connection with Applicants' § 1.129(a) Request is provided therein.

* * * * *